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LIFE CYCLE, DEVELOPMENT, AND CULTURE OF *XYLEBORUS GLABRATUS* (COLEOPTERA: CURCULIONIDAE: SCOLYTINAE)

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ABSTRACT

The redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), is a wood-boring pest that transmits the fungal pathogen *Raffaelea lauricola*, the causal agent of laurel wilt disease in American Lauraceae. This study documents the gallery formation patterns of *X. glabratus* as well as its life cycle and development at 25 ± 2 °C in logs of 3 natural hosts: avocado (*Persea americana*), redbay (*P. borbonia*) and swampbay (*P. palustris*). Females were observed to excavate galleries perpendicular to the tree trunk; galleries were characterized by a main entrance tunnel, from which branched secondary tunnels that, in turn, gave rise to tertiary tunnels. By dissecting infested logs daily, the length of time was determined for each developmental stage, and found to be comparable in all 3 hosts. Eggs were first encountered in avocado, redbay, and swampbay at 7, 11, and 10 days after gallery initiation (agi), respectively; larvae at 14, 20, and 14 days agi; pupae at 24, 26, and 26 days agi; and teneral adults at 31, 30, and 27 days agi. Despite comparable rates of development in all hosts, there were fewer progeny per female produced in avocado. Oviposition by the founding female extended over a broad time-span, and all stages were observed in the gallery at 1 month agi. Three larval instars were present, with mean head capsule widths of 0.21, 0.26, and 0.37 mm, respectively. Long term rearing of *X. glabratus* was achieved on swampbay logs soaked in water prior to infestation. Emergence of new females from logs was first observed at 60 d agi, indicating that teneral adults remain in hosts for ~1 month prior to dispersal. Emergence continued for up to 240 days, with maximum emergence observed between 120-150 days agi.

Key Words: *Persea americana,* avocado, redbay ambrosia beetle, rearing, laurel wilt

RESUMEN

El gorgojo de ambrosia del laurel rojo, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), es una plaga barrenadora de madera que transmite el hongo *Raffaelea lauricola*, el cual es el agente causal del secamiento del laurel en lauráceas Americanas. En este studio se documentan los patrones de formación de galerias, el ciclo biológico y el desarrollo de *X. glabratus* en maderos de tres hospederos : aguacate (*Persea americana*), laurel rojo (*P. borbonia*) y laurel de la ciénega (*P. pallustris*), a una temperatura de 25±2°C. Las hembras del gorgojo cavan galerias perpendiculars a el tronco; las galerias se caracterizan por un tunel principal el cual se divide en tuneles secudnarios y estos a su vez se dividen en tuneles terciarios. Al disectar estos troncos diariamente, se calculó el tiempo de desarrollo de cada estado del insecto, y se encontró que era similar en las tres plantas hospederas. Los huevos se encontraron por primera vez a los 7, 11 y 10 dias después de haber iniciado la primera gallería (ddilg) en aguacate, laurel ojo y laurel de la ciénega, respectivamente. Las larvas se encontraron a los 14, 20 y 14 días ddilg; pupas a los 24,26 y 26 días ddilg ; y los adultos recién formados a los 31, 30 y 27 días ddilg, en aguacate, laurel rojo y laurel de la ciénega, respectivamente. A pesar de esto se encontró que había menos progenie por hembra en aguacate que en los otros hospederos. La oviposición de la hembra fundadora de la colonia se produjo por un largo tiempo, y todos los estados de desarrollo fueron observados en las galerias un mes ddilg. Las larvas tienen tres instares, los cuales tienen un promedio de ancho de cápsula cefálica de 0.21, 0.26 y 0.37 mm en cada hospedero respectivamente. *Xyleborus glabratus* fué criado por un largo tiempo al utilizar troncos de laurel de la ciénega que habian estado sumergidos en agua antes de la infestación. La emergencia de nuevas hembras a partir de estos troncos ocurrió 60 días ddilg, indicando que los adultos recién formados permanecen en

los hospederos por un mes antes de dispersión. La emergencia del insecto continuó por 240 d, obteniendose un máximo de emergencia entre 120-150 dias ddilg

Palabras Clave: *Persea Americana,* aguacate, gorgojo de ambrosia del laurel rojo, crianza, secamiento del laurel

The redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), is an Asian species recently introduced into North America. It was first detected in 2002 near Savannah, Georgia (Rabagalia et al. 2006), and by 2004 its association with laurel wilt disease had been recognized (Fraedrich et al. 2008). *Xyleborus glabratus* is a minute wood-boring beetle with females 2.1-2.4 mm in length, 3 times as long as wide and dark brown to black in color. Males are rare, flightless, 1.8 mm in length, and 2.5 times as long as wide (Rabagalia et al. 2006). It is thought that *X. glabratus* was introduced via solid wood packing material, and the species constitutes the twelfth non-native ambrosia beetle known to become established in the United States since 1990 (Mayfield & Thomas 2009). Since its introduction a decade ago, *X. glabratus* has invaded 6 states in the southeastern Coastal Plain, including North Carolina, South Carolina, Georgia, Florida, Alabama, and Mississippi (USDA-FS 2012). The beetle is native to India, Japan, Myanmar, and Taiwan, but in these countries *X. glabratus* is not associated with tree disease (Beaver & Liu 2010) and appears to be a host generalist, since it has been recorded from a variety of plant families: Lauraceae [*Lindera latifolia* Hook. f., *Litsaea elongata* (Nees) Benth. et Hook. f., and *Phoebe lanceolata* (Wall. ex Nees) Nees]; Dipterocarpaceae (*Shorea robusta* C. F. Gaertn); Fagaceae [*Lithocarpus edulis* (Makino) Nakai]; and Fabaceae [*Leucaena glauca* (L.) Benth.] (Rabaglia et al. 2006).

Raffaelea lauricola T.C. Harr. Fraedrich & Aghayeva is the primary fungal symbiont of *X. glabratus* and the confirmed etiologic agent of laurel wilt disease in American Lauraceae (Fraedrich et al. 2008; Hanula et al. 2008). Tree species thus far identified as susceptible to laurel wilt include avocado (*Persea americana* Mill.), redbay [*P. borbonia* (L.) Spreng.], swampbay [*P. palustris* (Raf.) Sarg.], silkbay (*P. humilis* Nash), sassafras [*Sassafras albidum* (Nutt.) Nees], pondspice [*Litsea aestivalis* (L.) Fernald], pondberry [*Lindera melissifolia* (Walter) Blume], Northern spicebush [*Lindera benzoin* (L.) Blume], camphor tree [*Cinnamomum camphora* (L.) J. Presl], and California bay laurel [*Umbellularia californica* (Hook & Arn.) Nutt.], but additional Lauraceae are potentially at risk in Mexico, Central and South America, and the Caribbean Basin (Fraedrich et al. 2008; Mayfield et al. 2008; Smith et al. 2009a, 2009b; Peña et al. 2012; Mayfield et al. 2013). Laurel wilt disease is responsible for high mortality of native *Persea* species and for the death of backyard and commercial avocado trees in Florida (Carrillo et al. 2012, FDACS 2012). Consequently, the beetle and its fungal symbiont are considered a serious threat to both native forest ecosystems and commercial avocado production (Crane et al. 2008; FDACS 2012). To date, *X. glabratus* is the only confirmed vector of this pathogen, but other Scolytinae may be involved (Carrillo et al. 2012). The laurel wilt epidemic, including our current understanding of the mycopathogen, the beetle vector, and the susceptible host trees has been the subject of a recent review (Kendra et al. 2013).

Behaviorally, populations of *X. glabratus* established in the United States are atypical for ambrosia beetles in the tribe Xyleborini, particularly with respect to host selection. The beetle is capable of attacking live, apparently healthy trees, and thus far appears to be restricted to hosts within the Lauraceae. Most ambrosia beetles are generalists that colonize dead or moribund trees, and as a result, are attracted to ethanol (Miller & Rabaglia 2009), a semiochemical indicative of tree decay; however, since *X. glabratus* is a primary colonizer, it is not attracted to ethanol (Hanula et al. 2008, 2011). The only known long-range attractants for *X. glabratus* are host-based volatiles (kairomones), primarily sesquiterpenes (Hanula & Sullivan 2008; Kendra et al. 2011, 2012b, 2012c). Females of *X. glabratus* have a unimodal dispersal peak (Brar et al. 2012), engaging in host-seeking flight during the late afternoon, several h earlier than other species of *Xyleborus* [e.g., *X. ferrugineus* (Fabricius) and *X. affinis* Eichhoff] (Kendra et al. 2012a, 2012b). Since *X. glabratus* is not an economic pest in its native range, the species has not been studied well. To better understand the vector-pathogen-host complex, and to make informed decisions regarding management strategies, knowledge of the *X. glabratus* life cycle and development in U.S. hosts is required. Here we report for the first time (1) the life cycle and development of *X. glabratus* using redbay, swampbay, and avocado hosts under controlled laboratory conditions, (2) the characteristics of the gallery pattern excavated by a colonizing female, and (3) a method for long-term rearing of *X. glabratus* using conditioned host logs.

Materials and Methods

Insects

Redbay trees with high infestations of *X. glabratus* were collected from Austin Cary Memorial

Forest, Alachua County, Florida and Ordway-Swisher Biological Station, Putnam County, Florida to provide initial insects for laboratory investigations. The trunks of infested trees were cut at the base and sectioned into 40-45 cm logs. Logs were transferred immediately to the laboratory and 4-6 logs were placed in a beetle-emergence container. Emergence containers consisted of 32-gallon (121-L) refuse containers with tightly fitting lids (Rubbermaid Roughneck, Pleasant Prairie, Wisconsin), with a clear plastic collection cup attached to the side of the container near the neck. Each collection cup was partitioned into 2 compartments using plankton netting (150 micron, BioQuip Products, Rancho Dominguez, California). A manuka oil lure (Synergy Semiochemicals Corp., Burnaby, British Columbia, Canada) was placed in the lower compartment of the collection cup and replaced every 14 days, based on experimental determinations of attraction efficacy for this lure (Kendra et al. 2012c). Adult beetles were collected in the upper compartment of the cup which contained a moist paper towel to maintain high humidity. The permeable partition allowed movement of attractant volatiles released from the manuka lures, but prevented the beetles from direct contact with the lure. The containers were placed on their sides on a wire-frame shelf unit, such that the collection cups were facing downwards. A total of 20 beetle-collection containers were maintained in 2 rearing rooms at the Department of Entomology and Nematology, University of Florida, Gainesville, Florida. Rearing rooms were maintained at 25 ± 2 °C in complete darkness. Beetles were collected daily, with fully sclerotized (dark brown to black) *X. glabratus* females sorted and used for developmental studies.

Life Cycle and Development

Life cycle and developmental studies of *X. glabratus* were conducted using artificially infested logs of redbay, avocado, and swampbay. Logs of avocado cv. 'Booth 7' (Guatemalan-West Indian hybrid) were obtained from the University of Florida Tropical Research and Education Center, Homestead, Florida. A certified municipal arborist in Volusia County, Florida provided samples of redbay and swampbay, and the species identification was confirmed at the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, Florida. All logs were cut from healthy trees with no symptoms of laurel wilt nor visible signs of *X. glabratus* attack. Developmental studies in avocado were conducted during Sep-Oct 2010, in redbay during Mar-Apr 2011, and in swampbay during May-Jun 2011.

Logs of 4.5-6.5 cm diam. were cut into 8-10 cm lengths and then soaked in tap water for 48 h. For each host, 150 logs were used. To maintain moisture content, each log was kept standing upright in water in a 946 mL clear plastic container (American Plastics, Gainesville, Florida) throughout the experiment; the exposed water surface was covered with plankton netting (as above) to exclude test insects. To infest logs, 20 adult females were placed on the bark of each log and allowed to bore. Logs were kept in an incubator (Precision® illuminated incubator, Thermo Fisher Scientific, Waltham, Massachusetts) at 25 ± 2 °C in complete darkness. Each day, 3 logs were selected at random, split into small longitudinal pieces to expose beetle galleries, and each gallery searched thoroughly for insects. The duration of each study was approximately 40 days or until the teneral (newly emerged, light brown) adult stage was observed. Data recorded from each log consisted of the number of successful borings, the gallery pattern, and the number of each developmental stage. Boring was considered successful if, after removal of the outer bark, the gallery extended though the vascular cambium and into the underlying pith (dead xylem or heartwood).

Description of Life Stages

Throughout the developmental study outlined above, specimens of *X. glabratus* in the egg, larval, and pupal stages were collected and preserved in 70% ethyl alcohol. Photo-documentation and morphological descriptions were taken for each stage, and the number of instars was determined by measuring head capsule width with an ocular micrometer under a binocular microscope. To ascertain measurements from the first instars, eggs were collected from galleries 20 d after infestation. Eggs were placed individually on moist tissue in a Petri dish (50 × 9 mm, BD Falcon, Franklin Lakes, New Jersey), and head capsule width was measured upon larval emergence.

Gallery Pattern

Redbay logs (5-6 cm diam.) heavily infested with *X. glabratus* were collected from Ordway-Swisher Biological Station. Entry holes of *X. glabratus* were initially identified and marked based on size (0.8 mm diam.) (Hanula et al. 2008; Mayfield & Hanula 2012), and then confirmed by presence of an adult female within the gallery. In line with an entrance hole, logs were dissected horizontally (cross-sectioned) with a miter saw to expose the gallery system. Galleries were then traced on transparency sheets and the structure and pattern of the gallery were described.

Culture on Swampbay Logs

To develop laboratory rearing methods for *X. glabratus,* and to obtain initial data on reproductive potential, time of adult emergence (disper-

sal), and longevity of rearing substrates, beetle colonies were established on freshly collected, healthy logs of swampbay. A total of 34 logs [mean (± SE) dimensions of 9.5 (± 1.6) cm length × 5.9 (± 0.2) cm diam.] were soaked in tap water for 48 h, blotted dry, and placed upright into individual 946 mL clear plastic containers (American Plastics) containing 100 mL of water. As before, plankton netting was used as a barrier above the water, and 20 females were placed on each log. Logs were kept in an incubator (as above) and water level was maintained throughout the experiment. The number of females emerging per log was counted at intervals of 7-14 days, and the duration of the experiment was 240 days, conducted during Apr-Dec 2011.

Statistical Analysis

SAS procedures were used to perform all statistical analyses (SAS Institute 2004). Data for successful boring with the 3 hosts were analyzed using Proc GLIMMIX (response variable = successful boring, response distribution = binomial, fixed effects = different hosts). Number of individuals per developmental stage observed in different hosts were compared using Proc GLIMMIX (response variable = number of individuals per developmental stage, response distribution = poisson, fixed effects = different hosts, random effects = time of observation). Monthly emergence from the rearing study was analyzed using Proc GLM with response variable as monthly emergence, and days as fixed effects, $N = 34$. Means were separated using the Tukey-Kramer multiple comparisons test. Regression analysis was used to evaluate the relationship between instar and head capsule width. We applied Dyar's rule in the form of a linear regression model Log y = a + bx, where y = head capsule width, and x = instar (Dyar 1890; Klingenberg & Zimmerman 1992).

Results

Life Cycle and Development

In avocado logs, eggs were first observed at 7 days, larvae at 14 days, pupae at 24 days, and teneral adults at 31 days after gallery initiation (agi) (Fig. 1A). In redbay, those sequential stages were observed at 11, 20, 26, and 30 d agi (Fig. 1B); and with swampbay, the corresponding observations were made at 10, 14, 26, and 27 d agi (Fig. 1C). Since developmental times were similar with all 3 hosts, data were combined to construct a generalized life cycle for *X. glabratus* at 25 °C in *Persea* hosts. Mean ± SE pre-oviposition period was 9.3 ± 1.1 d; and duration of the egg, larval, and pupal stages were 6.6 ± 1.4 d, 9.3 ± 1.7 d, and 5.0 ± 0.8 d, respectively. All 4

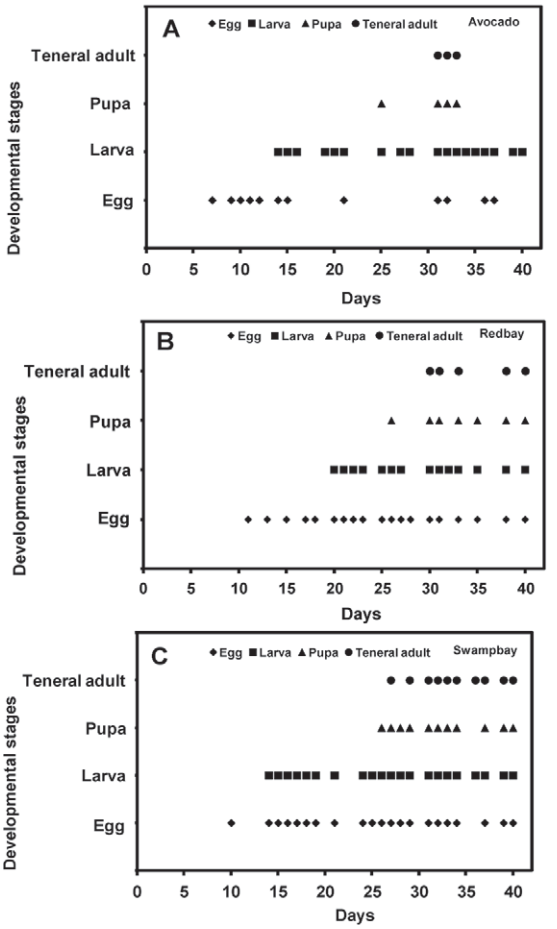

Fig. 1. Presence of *Xyleborus glabratus* in logs from host trees at 25 ± 2 °C and 24 h dark conditions. Graphs depict observations made daily for 40 days of developmental stages in (A) avocado, *Persea americana*, (B) redbay, *P. borbonia*, and (C) swampbay, *P. palustris.*

developmental stages were found concurrently from about day 30 and thereafter (Fig. 1). Since eggs could be observed on most consecutive days, especially with redbay (Fig. 1B) and swampbay (Fig 1C), it appears as though oviposition in *X. glabratus* is fairly continuous, rather than in discrete batches. There were significant differences among hosts with respect to percent successful boring ($F_{2, 287} = 10.35$; $P < 0.0001$) (Table 1), with less boring in redbay and swampbay logs compared to avocado logs (Table 1). There were significant differences among the numbers of eggs ($F_{2, 287} = 43.19$; $P < 0.0001$), larvae ($F_{2, 287} = 75.68$; $P < 0.0001$), pupae ($F_{2, 287} = 54.84$; $P < 0.0001$) and teneral adults ($F_{2, 287} = 45.72$; $P < 0.0001$) observed in redbay, swampbay and avocado. Highest numbers of individuals in each developmental stage were observed in swampbay (Table 1).

TABLE 1. DEVELOPMENT OF *XYLEBORUS GLABRATUS* IN LOGS OF 3 SPECIES OF *PERSEA* UNDER CONTROLLED LABORATORY CONDITIONS FOR 40 DAYS.

Host	Percent successful boring (Mean ± SE)	Mean No. ± SE of individuals per developmental stages collected per log			
		Egg	Larva	Pupa	Adult
Avocado $N = 118$	50.0 ± 0.1 a	0.5 ± 0.1 b	1.2 ± 0.4 b	0.2± 0.1 c	0.1 ± 0.1 b
Redbay $N = 120$	41.8 ± 0.1 b	0.5 ± 0.1 b	1.1 ± 0.3 b	0.3± 0.1 b	0.3 ± 0.1 b
Swampbay $N = 93$	45.0 ± 0.1 b	1.7 ± 0.3 a	3.3 ± 0.5 a	1.4 ± 0.3 a	1.1 ± 0.3 a

Means followed with same letter are not significantly different based on Tukey-Kramer test for difference of means ($P < 0.05$).

Developmental Stages

Egg: White, translucent and ovoid. Mean (± SE) length and width were 0.63 (±0.004) and 0.27 (±0.003) mm, respectively (n=44). Larva: Legless, dull whitish in color with head capsule white. Measurements of larval head capsule showed 3 peaks (Fig. 2), indicating 3 instars, and there was no difference in head capsule size based on developmental host (Fig. 3, Table 2). Linear regression of head capsule data (applying Dyar's rule of geometric progression of capsule width with successive instars) yielded high R^2 values (Fig. 3), supporting our conclusion of 3 instars for *X. glabratus*. Pupa: white, exarate, typical of that reported for other Scolytinae.

Gallery Pattern

Females of *X. glabratus* excavate the gallery by pushing out the macerated woody tissue, which gives rise to distinctive sawdust sticks at the entrance hole. The gallery system is constructed perpendicular to the trunk, in a horizontal plane, and consists of a primary entrance tunnel that, over time, branches into 2-5 secondary tunnels, from which 0-3 tertiary tunnels may also branch (Fig. 4). In redbay logs with a diam of 5-6 cm, the mean (± SE) length of a primary tunnel was 8.5 (± 0.8) mm (n = 24). The mean (± SE) gallery length and width recorded was 32.1 (± 2.0) and 28.0 (± 2.1) cm, respectively (n = 24). Eggs were observed at the distal ends of secondary and tertiary tunnels, in groups of 1-8, indicating that these portions of the tunnel system function as brood galleries. This was also the site where pupae were observed.

Culture on Swampbay Logs

Xyleborus glabratus was reared successfully on pre-soaked swampbay logs. Over the period of study, a total of 1,947 beetles emerged from 34 logs, with mean ± SE emergence per log equaling 57.3 ± 5.7 females. Beetle emergence was first observed at 60 d agi, and maximum emergence was seen between 120-150 d agi. Emergence continued up through 240 d agi, at which time the experiment was terminated (Fig. 5). It is likely that there were overlapping generations of beetles developing within these logs. Since observations were made at 7-14 d intervals, it was not possible to assess if newly emerged females reinfested the same logs.

DISCUSSION

Redbay and swampbay are the 2 ecologically important trees in the family Lauraceae that have been severely affected by laurel wilt, with over 90% mortality reported in some infested areas (Fraedrich et al. 2008). The disease has killed numerous backyard avocado trees throughout Florida (Carrillo et al. 2012), and in the spring of 2012 laurel wilt was detected in the commercial avocado production areas of Miami-Dade County, FL (FDACS 2012). We conducted controlled laboratory studies to investigate and compare the development of *X. glabratus* in these 3 primary hosts. Host tree species (and physiological state of an individual tree), is likely to affect the suitability of wood as a substrate for fungal growth. The mycelium consumed by ambrosia beetles derives nutrition from materials stored within the wood, primarily dead xylem tissue (Panshin & De-Zeeuw 1977; McIntosh 1994). In addition to nutritional quality, fungal growth will also depend upon favorable temperature, levels of respiratory gases, moisture content, and other physical/chemical properties of the host internal environment (Rudinsky 1962). Although similar boring and development times were observed with the 3 hosts evaluated in this study, there were differences in the number of *X. glabratus* progeny produced. Our results indicate that avocado may be a less suitable reproductive host than swampbay, a finding consistent with results reported by Carrillo et al. (2012). Since our laboratory study used destructive sampling, follow-up studies are needed with live trees to better understand the tritrophic interaction among host species, insect vector, and fungal symbiont under natural field conditions.

In terms of insect behavior, field tests have found similar attraction of *X. glabratus* to cut

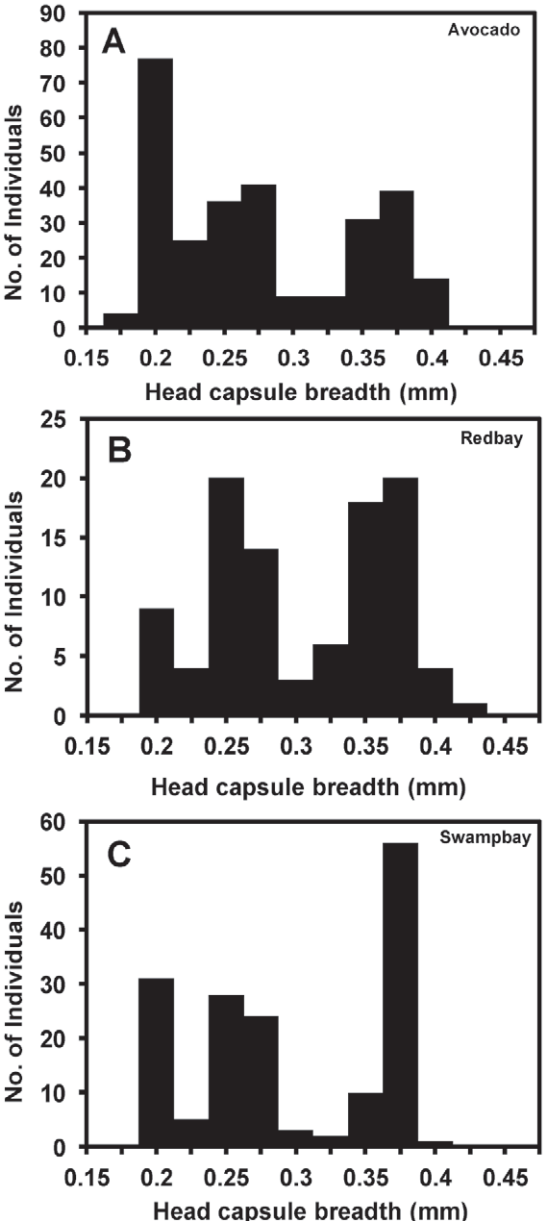

Fig. 2. Frequency distribution of head capsule width measurements from larvae of *Xyleborus glabratus* reared in (A) avocado, *Persea americana* (n = 255), (B) redbay, *P. borbonia* (n = 100), and (C) swampbay, *P. palustris* (n = 157).

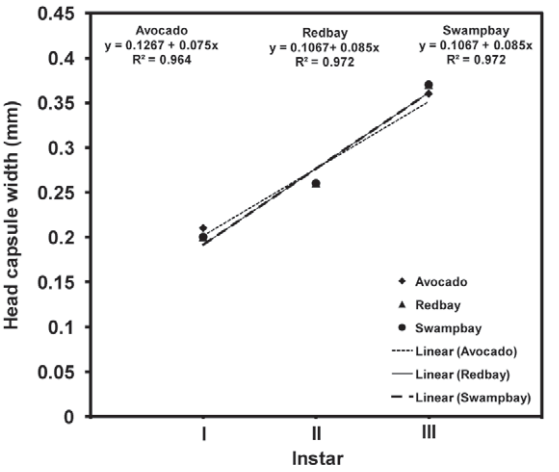

Fig. 3. Linear regression of mean head capsule width as a function of larval instar for *Xyleborus glabratus*.

bolts of redbay, swampbay, and avocado (Hanula et al. 2008; Kendra et al. 2013). With avocado bolts representative of the 3 horticultural races (Mexican, Guatemalan, and West Indian), there was no significant difference in attraction of *X. glabratus* in field tests; and in laboratory bioassays, a high percentage of females (~80%) readily bored into all 3 cultivars (Kendra et al. 2011). Avocado emits the same sesquiterpene kairomones as redbay and swampbay (Niogret et al. 2011), and the combined results of these studies suggest that avocado is just as likely to be attacked as native *Persea* species, even though it may not be the best host for reproduction. Beetle reproduction is not required for transmission of the pathogen, only host recognition and successful boring. However, once the optimal reproductive hosts (i.e., swampbay trees) become scarce in south Florida, one would assume there would be strong selection for beetles capable of successful reproduction in avocado. It remains to be seen how the epidemiology of laurel wilt disease in avocado groves will compare to that played out in U.S. forest ecosystems.

The rate of development observed for *X. glabratus* in this study is similar to that reported in the literature for other species of *Xyleborus*. For example, our generalized model estimates the average developmental time for egg, larval, and pupal stages of *X. glabratus* to be 6.6, 9.3, and 5.0 d; and the corresponding stages in *X. ferrugineus* reared on artificial diet have been observed to take 4.5, 8.1, and 4.6 days (Kingsolver & Norris 1977a). Likewise, other congeneric species examined are known to have 3 larval instars, e.g., *X. ferrugineus* (Norris & Chu 1985) and *X. celsus* Eichhoff (Gagne & Kearby 1979), and extended periods of oviposition leading to overlapping generations, e.g., *X. pfeili* (Ratzeburg) (Mizuno & Kajimura 2002). Of particular note in our study is that teneral adults were observed in dissected galleries at ~30 days agi, but adults did not emerge from intact logs until ~60 days agi. This observation indicates that female *X. glabratus* remain within their natal trees for an extended period of time prior

TABLE 2. HEAD CAPSULE WIDTH MEASURED FOR THE 3 LARVAL INSTARS OF *XYLEBORUS GLABRATUS* REARED IN LOGS OF 3 SPECIES OF *PERSEA*.

Host	Instar no.	No. of larvae	Width of head capsule (mm)			X_{n+1}/X_n
			Mean (X)	SE	Range	
Redbay						
	I	13	0.21	0.003	0.20 - 0.22	1.30
	II	34	0.26	0.002	0.25 - 0.27	1.42
	III	48	0.37	0.002	0.35 - 0.40	
Swampbay						
	I	36	0.21	0.001	0.20 - 0.22	1.30
	II	52	0.26	0.002	0.25 - 0.27	1.42
	III	69	0.37	0.001	0.35 - 0.40	
Avocado						
	I	101	0.21	0.001	0.20 - 0.22	1.24
	II	78	0.26	0.001	0.25 - 0.27	1.40
	III	66	0.36	0.001	0.35 - 0.40	

X_{n+1}/X_n = Mean of subsequent larval stage/Mean of previous larval stage

to dispersing. That 'lag' time may be required for full sclerotization of the cuticle, sexual maturation, mating with sibling males, garnering fungal spores (conidia) within mandibular mycangia, and procuring adequate energy stores necessary for engaging in host-seeking flight. In essence, females must 'fast' after leaving the natal tree, going without food during the span of dispersal flight and initial gallery formation until symbiotic fungal gardens can be cultured within new host trees.

The extensive tree-like branching patterns we documented for the galleries of *X. glabratus* are not unlike those reported for other *Xyleborus*; however, there are species-specific differences as to the location of the brood galleries (Kajimura & Hiji 1994; Kingsolver & Norris 1977a). Gallery size has been shown to be an important factor in determining fitness of ambrosia beetles. In both *Xylosandrus mutilatus* Blandford (Karimura & Hijii 1994) and *Xyleborus pfeili* (Mizuno & Kajimura 2002), there is a positive correlation between overall gallery length and number of offspring. This implies that increased gallery length results in increased fungal growth, and that successful brood development is directly related to the quantity of symbiotic fungus (Kingsolver & Norris 1977b). In other words, it is adaptive for ambrosia beetles to construct extensive gallery networks within host trees, and this requires a host of large diam. With *X. glabratus*, field surveys of infested swampbay trees indicate that host-seeking females have a 'diameter preference'; there is a strong positive correlation between diam of host tree and density of beetle entrance holes (Kendra et al. 2013). Assessment of host diam by females may potentially be obtained from visual cues, from proximo-distal distributions of attractive sesquiterpenes (Niogret et al. 2013), or from both. Regardless, the consequence of this behavior is that the oldest (largest diam) trees are typically the first to be attacked, and over the next few years, the vector colonizes progressively smaller diam trees until the site is depleted (Kendra et al. 2013).

CONCLUSION

The redbay ambrosia beetle is firmly established in the southeastern U.S. where it vectors *Raffaelea lauricola*, the fungus responsible for laurel wilt, a lethal disease that impacts both forestry and agriculture. Its range continues to expand, and despite intensive research over the past few years, no efficacious and economical means of control have been identified. Management of laurel wilt disease and its insect vector will require a holistic approach, which is contingent upon a better understanding of the insect vector, its symbiotic fungus, and the susceptible host Lauraceae. This publication reports a laboratory rearing method to facilitate experimental research on *X. glabratus*. It also provides information on the basic biology, life cycle, and developmental stages of the pest on the 3 primary hosts impacted in the state of Florida.

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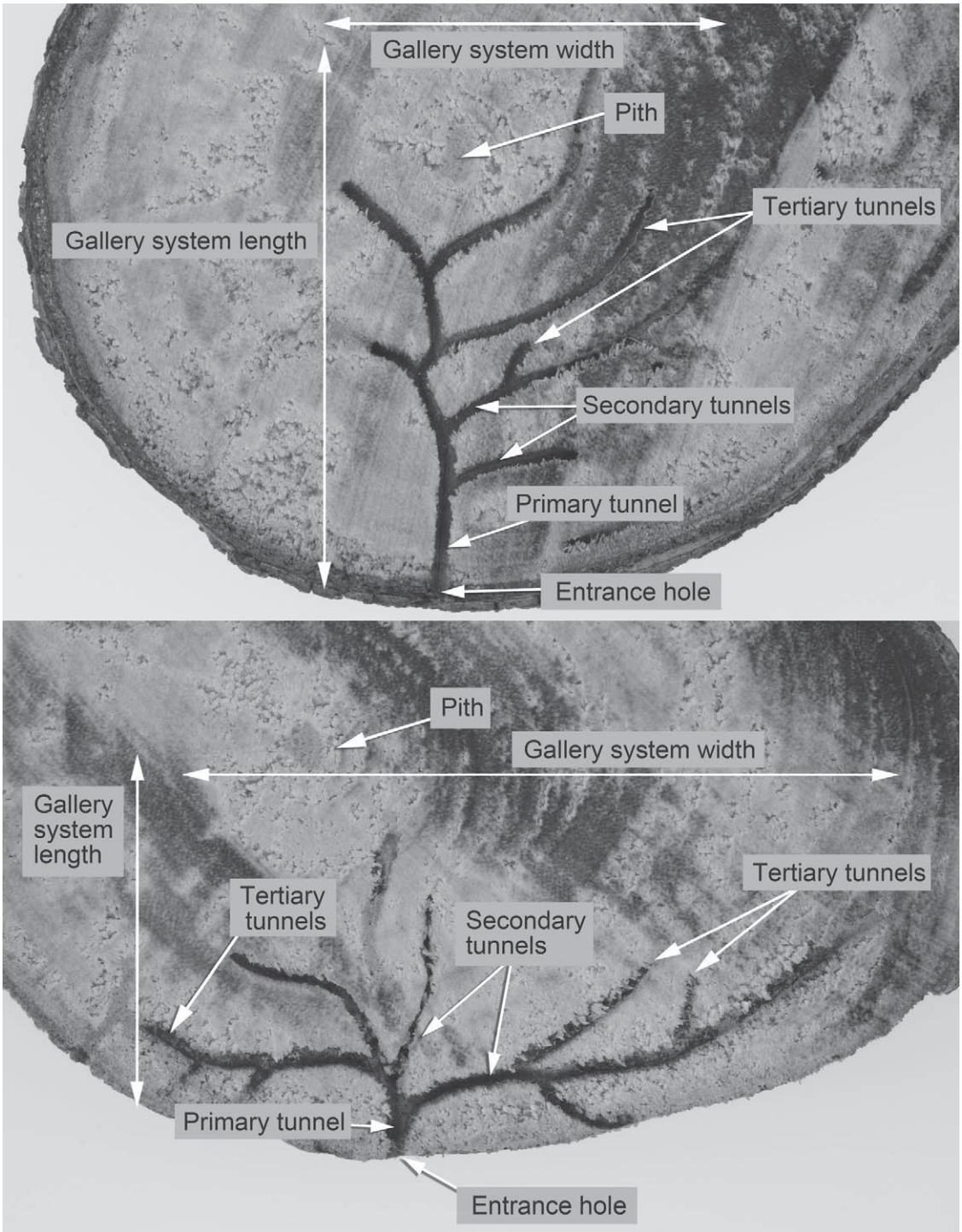

Fig. 4. Two examples of gallery patterns excavated by female *Xyleborus glabratus* in redbay, *Persea borbonia*.

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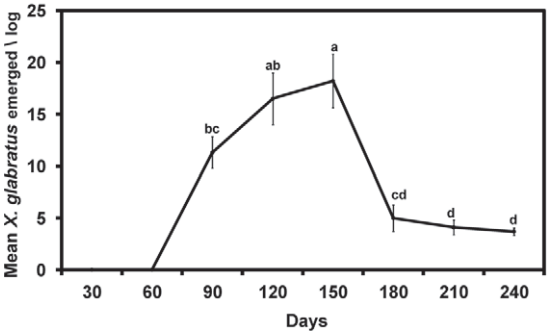

Fig. 5. Emergence (mean ± SE) of adult female *Xyleborus glabratus* from logs of swampbay, *Persea palustris* in which they were reared at 25 ± 2 °C and 24 h dark conditions over a period of 240 days (n = 34). Means followed by the same letter are not significantly different based on Tukey-Kramer test for difference of means ($P < 0.05$; $F_{5,165} = 19.26$; $P < 0.0001$).

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